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Original Article

Synthesis and Investigation of Cobalt-Containing Phthalocyanine Pigment

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Abstract - *In this study, the synthesis of cobalt phthalocyanine dye pigment was carried out, and the corresponding reaction equation for its formation was provided. Also, density functional theory calculations based on quantum chemical parameters of the isoindole ligand in the cobalt phthalocyanine complex were performed to determine the electronic properties of the complex. The synthesized cobalt phthalocyanine pigment was thoroughly characterized by thermal analysis and Raman spectroscopy. Thermal analysis revealed the pigment's thermal stability and decomposition temperatures, while Raman spectroscopy provided information about its molecular structure and chemical bonding. Furthermore, the optical properties of the pigment, such as absorption and photoluminescence spectra in various solvents, were investigated to evaluate its light absorption ability and emission intensity. In addition, the current and voltage values obtained by solar cells sensitized with a natural pigment based on raspberry fruit extract and a dye based on cobalt phthalocyanine pigment were compared for 10 days. As a result, it was noted that the obtained solar cell based on cobalt phthalocyanine pigment is resistant to aggressive environments and produces high power. The results indicate that the pigment is a promising material not only for industrial dyes but also for high-performance optical applications and dye-sensitized solar cells.*

Keywords - *Dye-sensitized solar cell, Phthalocyanine, Pigment, Thermal analysis, Raman spectrum, Optical absorption, Photochemical analysis.*

1. Introduction

One of the most effective solutions to today's energy problems is the use of solar energy. It is important to create cheap and promising solar cells based on organic semiconductor materials. Therefore, among organic semiconductor materials, phthalocyanine-based sunlight-sensitive semiconductor materials are of particular importance. Phthalocyanine-based molecular semiconductors have attracted significant scientific interest in recent years as promising materials for various applications in organic electronics, including light-emitting devices, solar energy conversion systems, and advanced nanoelectronic components [1, 2]. In phthalocyanine molecules, charge transport occurs through the movement of electrons and holes. Under the influence of energy and temperature, π-electrons in the aromatic system become activated via quantum transitions between energy levels and move in the direction of the applied electric field [3, 4]. The presence of a central metal atom in phthalocyanines enables their interaction with multielectronic organic components, significantly enhancing their overall electrical conductivity. Metal phthalocyanines demonstrate high sensitivity to chemical interactions and can serve as photoactive layers or coating materials in tandem solar cells, thereby facilitating efficient electron transfer processes. Developing organic semiconductor materials based on such structures and their effective application in organic solar cell technologies is considered a promising approach [5, 6]. One of the key advantages of phthalocyanine-based organic semiconductors lies in their high optical and electrical activity, the possibility of low-cost and simple synthesis methods, environmental safety, and economic efficiency. These features make such materials a valuable scientific and practical platform for emerging research and technological developments. Currently, metal phthalocyanine-based semiconductors are being successfully applied in the development of next-generation solar cells, energy storage devices, and as sensor materials for highly sensitive gas detectors [7, 8]. Phthalocyanine-based complexes possess efficient absorption capabilities in the visible and near-infrared regions of the electromagnetic spectrum. These materials are extensively utilized in the fabrication of complex semiconductor structures as well as in tandem solar cells that integrate the advantages of both organic and inorganic semiconductors. Thin films based on supramolecular structures derived from phthalocyanine and its derivatives have been successfully used as active layers or nanocomposite matrices in various nanoelectronic devices, including thin-film transistors, sensors, and photoconversion systems. The unique combination of optical, electrical, and chemical properties inherent in phthalocyanines plays an important role in their widespread use in modern materials science and nanotechnology applications. The most important and most studied representatives of the class of semiconducting polymers and data on phthalocyanine-based semiconductor materials are presented in Table 1. These polymers and complex macromolecular compounds exhibit high stability, resistance to aggressive environments, and excellent photo and electrical conductivity when combined with flexible substrates [9].

Phthalocyanines containing either metal or non-metal elements as central atoms can form complex metal-organic structures through the incorporation of heterocyclic compounds with phthalic anhydride fragments acting as ligands. The synthesis and investigation of novel structures represent a promising scientific direction in the advancement of semiconductor and photoanode materials. These compounds exhibit high chemical stability, resisting reactions with strong acids and bases, and demonstrate significant optical absorption within the infrared and visible spectral regions [10, 11].

Studies on the optical properties of phthalocyanine-based semiconductor supramolecular materials show that their photothermal conversion efficiency can reach 25%. Due to their excellent electrical conductivity, efficient light absorption, and excellent chemical and thermal stability, these phthalocyanine-based semiconductor pigments have attracted great attention in bioelectronics and biomedical applications. Composite semiconductor polymers introduce a novel approach within modern electrical engineering. Highly ordered crystalline structures with long-range molecular alignment exhibit enhanced charge carrier mobility.

Among the various classes of semiconductor materials, phthalocyanine-based semiconductor pigments are particularly promising for use in electronic devices. Their unique optical and electronic behaviors are associated with the existence of an energy gap between redox-active electronic states, delocalization of p-electrons, efficient transport mechanisms along supramolecular chains, and noncovalent bonds [12]. The semiconducting properties of metal phthalocyanine complex compounds are closely related to their electronic structure, energy levels of molecular orbitals, spatial distribution of charge density, and characteristics of electrostatic potential fields. These parameters are the main criteria determining their effectiveness as semiconductors. In particular, the energy differences (ΔE) between Highest Occupied Molecular Orbital (HOMO) and Lowest Unoccupied Molecular Orbital (LUMO) play a key role in assessing the electronic transition and conductivity potential of these systems. Therefore, the main problem in the analysis of the semiconducting properties of metal phthalocyanines in the scientific literature is that the electronic structure of metal phthalocyanines, energy levels of molecular orbitals, spatial distribution of charge density, and quantum-chemical properties have not been fully studied, which determines the relevance of research in this area [13, 14]. The purpose of this research work is to synthesize a semiconducting polymer dye pigment used as a dye pigment for dye-sensitized solar cells and to study its physicochemical, as well as volt-amper, optical, and semiconducting properties.

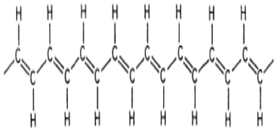
2. Materials and Methods

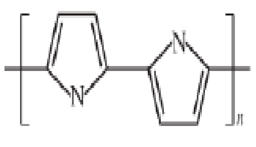
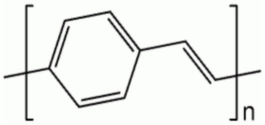
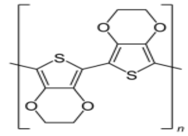
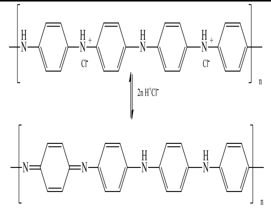
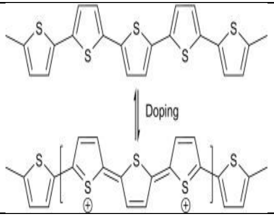
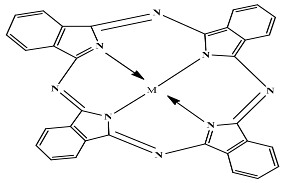
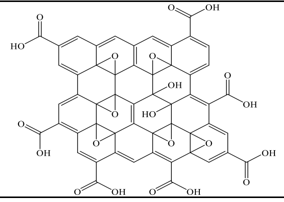
In this study, various instruments and analytical methods were employed. A Muffle furnace KEP25-1/1250P and a Japanese IR-Fourier spectrometer (Shimadzu IRAffinity-1) were used. The elemental composition was analyzed using a high-performance energy-dispersive X-ray fluorescence spectrometer (Japan). The thermal properties of the synthesized semiconducting cobalt phthalocyanine pigment were investigated using differential thermal and thermogravimetric analysis methods (DTG-60, simultaneous DTA-TG by SHIMADZU). Raman spectroscopy (HORIBA) was used to study the pigment's chemical composition and bonding nature. Furthermore, this research work used UV spectroscopy and analytical data measured on Щ300 and DT 9205A digital multimeters to study the optical and semiconducting properties of the newly synthesized cobalt-containing phthalocyanine pigment.

2.1. Materials

The following reagents were needed: phthalic anhydride, urea, cobalt salt, catalyst, a specially prepared transparent glass plate (on one side), titanium dioxide, zinc oxide, and light-sensitive dye pigments responsive to solar radiation.

Table 1. Overview of organic semiconducting polymers and organometallic materials [10, 15]

№	Names of semiconducting materials	Year of discovery	Structural formula	Optical absorption energy band gap (eV)	Conductivity S/cm-1
1	Polyacetylene	1977		1,5	10^3 - $1,7*10^5$

2	Polypyrrole,	1979		3,1	10^2 - $7,5*10^9$
3	Poly (phenylene vinylene)	1979		2,5	3 - $5*10^3$
4	Poly (3,4-ethylenedioxythiophene) (PEDOT),	1980		1,5	300
5	Polyaniline	1980		3,2	30-200
6	Polythiophene	1981		2,1	$10-10^3$
7	Phthalocyanines	1907		1,4	10 $^{-6}$ Sm^{-1} × m^{-1} 10^{-4} Sm^{-1} ×m $^{-1}$
8	Graphene and Graphene oxide	1859		1,4	2.98 * 10^4 Sm^{-1} 2 * 10^2 Sm^{-1}

3. Experimental Part

3.1. Preparation of Cobalt-Containing Phthalocyanine Pigment

Cobalt phthalocyanine pigment was synthesized using cobalt (II) sulfate, urea, phthalic anhydride, and a suitable catalyst. In a 250 ml heat-resistant beaker, 5.62 g (0.02 mol) cobalt (II) sulfate, 11.84 g (0.08 mol) phthalic anhydride, 9.6 g (0.16 mol) urea, and 0.2% (by total mass) sodium borohydride were added.

The resulting mixture was transferred to an oven heated to 300 °C and kept at this temperature for 3 hours. During the thermal process, the initially colorless mixture underwent chemical transformation, forming a brown, powdery product. After the reaction was complete, the solid product was removed from the oven, allowed to cool to room temperature, and then dissolved in concentrated sulfuric acid (98%) with constant stirring for 40 minutes.

In the final step, the brown powder, which was treated with acid, turned blue-green under the influence of an acidic environment and was washed repeatedly with distilled water and 10% ammonia solution and dried in a drying oven at 50 °C for 24 hours. A complex compound [$C_{32}H_{16}CoN_8$] (Figure 1) consisting of phthalic anhydride, cobalt (II) sulphate salt, urea, and ammonium heptamolybdate was synthesized, and its optical and semiconductor properties were studied using physicochemical methods.

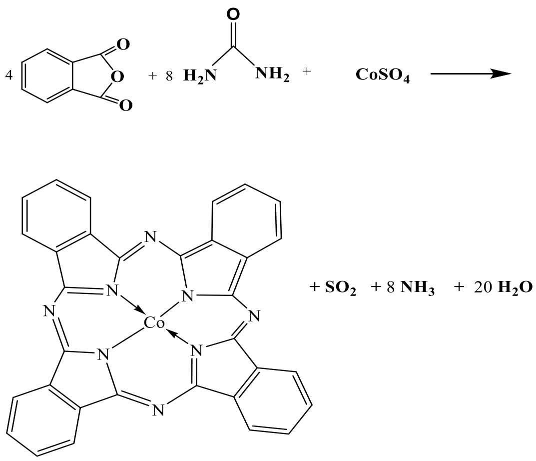


Fig. 1 Proposed reaction mechanism for the formation of cobalt phthalocyanine pigment

Subsequently, boiling water was gradually added to the solution until the total volume was restored, followed by additional stirring. This dilution step facilitated the dissolution and removal of unreacted starting materials into the liquid phase. Upon further purification, dissolution, and neutralization, dark blue crystals precipitated at the bottom of the beaker.

The solubility of the prepared cobalt phthalocyanine pigment in various organic solvents was investigated. As a result, it was found to have good solubility in solvents such as Dimethylformamide (DMF), Dimethyl Sulfoxide (DMSO), and N-Methylpyrrolidone (NMP). The overall yield of the reaction product was 82%.

4. Results and Discussion

4.1. ESP and Mullekin Charge Distribution of the Isoindole Ligand in the Formation of Cobalt Phthalocyanine Complex

The localization of the donor centers on the electrostatic surface allows us to characterize this ligand as a geometrically ordered and electronically active coordination system. It was noted that the electron density zones observed in the ESP map, in turn, are in full agreement with the spatial location of the HOMO-LUMO orbits and the Mulliken charge distribution. Such an agreement allows us to reliably interpret the electronic structure of the ligand molecule during complex formation, the nature of the donor centers, and their spatial activity on the basis of a single quantum-chemical model.

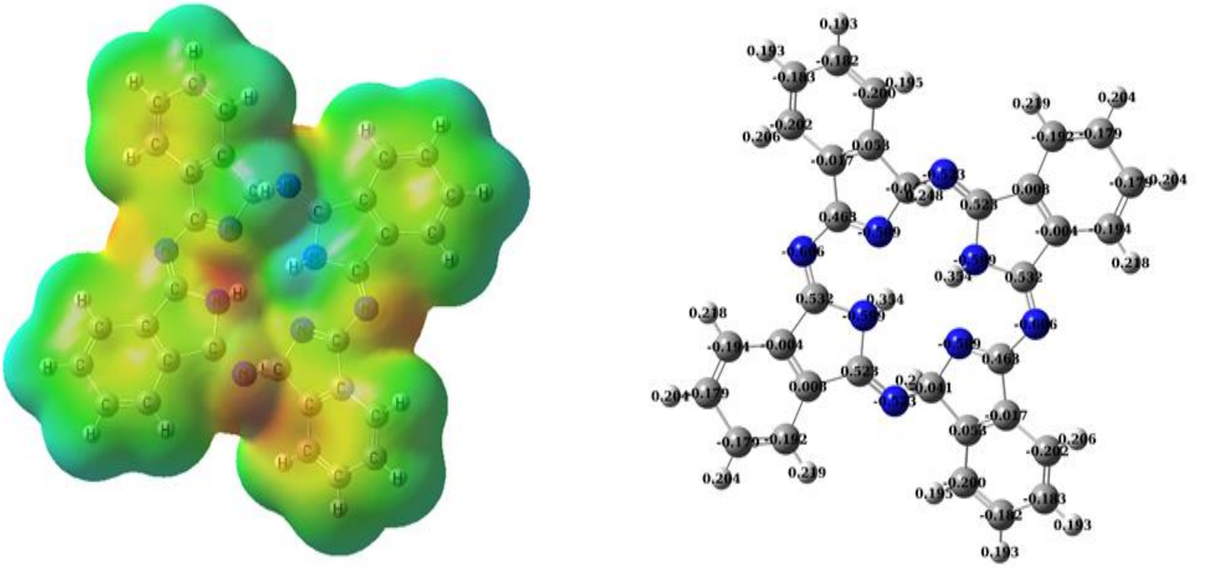


Fig. 2 ESP and Mullekin charge distribution of the isoindole ligand in cobalt phthalocyanine

The results show that this ligand is a molecule with multicenter, selective, and clearly distinguished donor centers in terms of electron density, which serves as a convenient basis for the formation of stable, energetically advantageous, and spatially directed coordination complexes with metal ions [16].

4.2. Raman Spectroscopic Analysis of the Cobalt-Containing Phthalocyanine Pigment

The Raman spectroscopic characterization of the cobalt-containing phthalocyanine pigment was conducted using a high-precision instrument manufactured by HORIBA Scientific (Japan). The obtained spectra allowed us to estimate several structural parameters, including the average interlayer or interatomic spacing, the orientation of individual crystals or molecules, the crystalline or amorphous nature of the material, and its quantitative distribution. In addition, the instrument helped to determine the crystallite size, shape, and internal structure, as well as the qualitative and quantitative composition of the substance. The analysis showed that the composition of the cobalt-containing phthalocyanine pigment was determined to consist of approximately 47.85% crystalline and 52.15% amorphous phases.

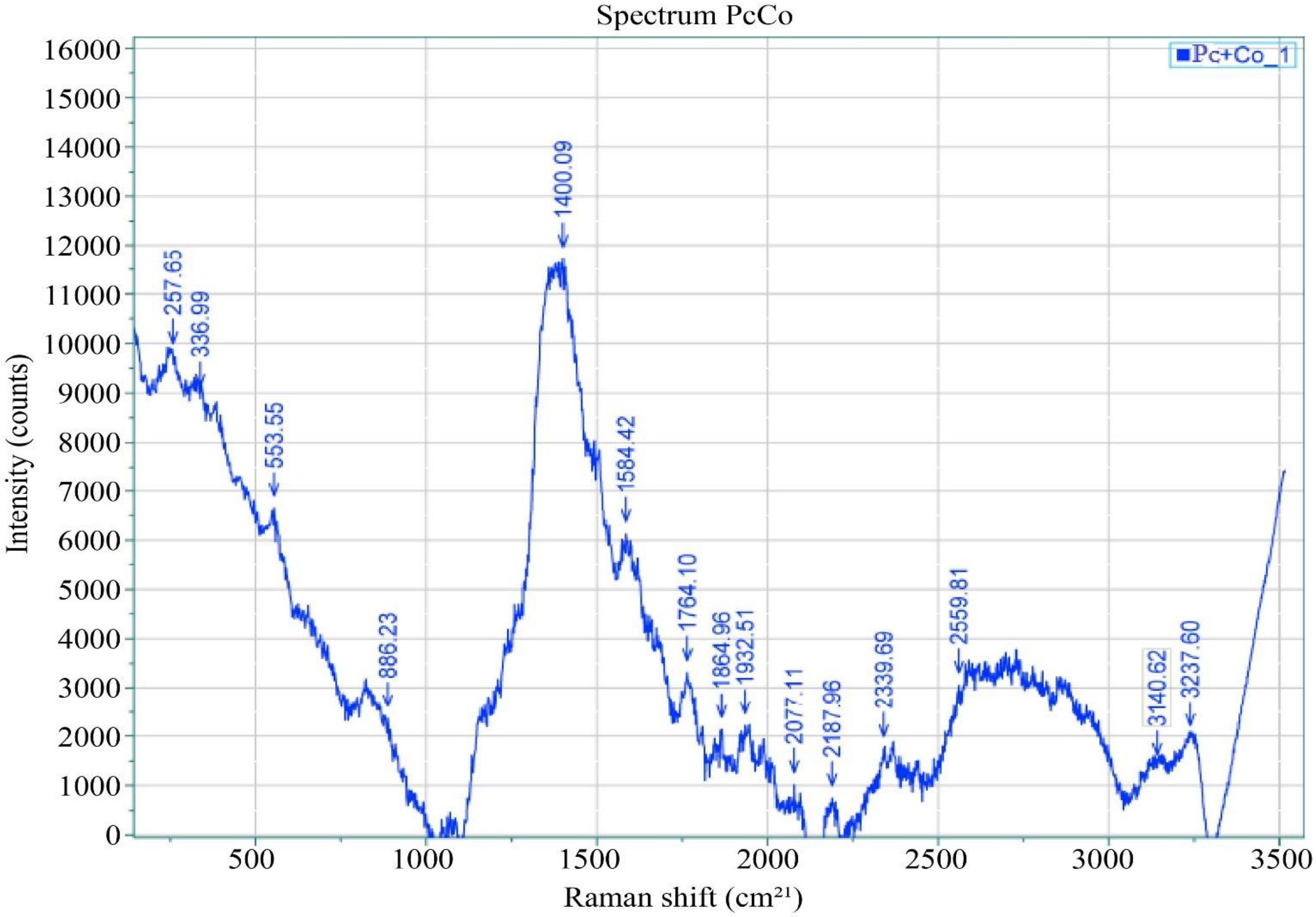


Fig. 3 Raman spectroscopic analysis of the cobalt-containing phthalocyanine pigment

The results of the Raman spectroscopic analysis led to the following conclusions regarding the composition of the pigment. The absorption band observed at 3140.62 cm⁻¹ indicates the presence of the aromatic group of the benzene ring.

Absorption at 3237.60 cm⁻¹ corresponds to N–H stretching vibrations, while the band at 2559.81 cm⁻¹ is attributed to O–H bonds involved in hydrogen bonding. Strong stretching and intense vibrational bands detected at 1764.83 cm⁻¹ are associated with C=C bonds.

The absorption peak at 1864.96 cm⁻¹ is related to C=O bonds, and the band at 1584.42 cm⁻¹ corresponds to differential vibrations of N–H bonds. The spectral region at 1400.09 cm⁻¹ is assigned to C–H bonds, whereas the band at 886.23 cm⁻¹ is indicative of metal-nitrogen (Me–N) differential vibrational modes [17].

4.3. Thermal Analysis of Cobalt-Containing Phthalocyanine Pigment

The thermal stability of the sorbent synthesized via solid-phase reaction of cobalt sulfate, urea, phthalic anhydride, and sodium borohydride was evaluated using Differential Thermal Analysis (DTA) and Thermogravimetric Analysis (TGA) with a SHIMADZU DTG-60 instrument (Japan). For the analysis, 5.485 mg of the powdered sample was placed in a porcelain crucible.

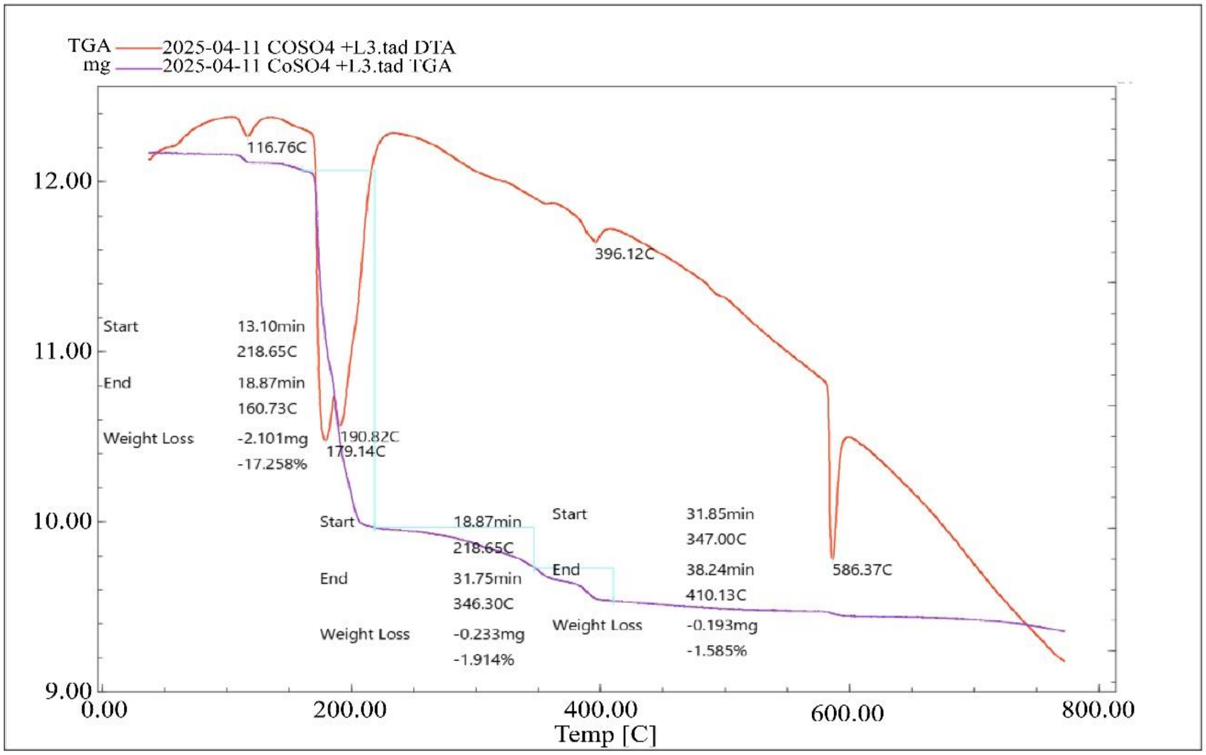


Fig. 4 Differential Thermal Analysis (DTA) and Thermogravimetric Analysis (TGA) of the cobalt-containing phthalocyanine pigment

The measurements were carried out under an argon atmosphere (gas flow rate: 80 mL/min), at a heating rate of 10°C/min, up to a maximum temperature of 800°C. The derivatogram was automatically recorded using the following sensitivity settings: T-800, TG-200, and DTA – 1/10 galvanometer sensitivity [18]. In the Differential Thermal Analysis (DTA) of the cobalt-containing phthalocyanine pigment, a single characteristic exothermic heat release was observed. The heat release began at 46.75 minutes into the analysis, corresponding to a temperature of 396.12°C, and ended at 31.85 minutes, when the temperature reached 586.37°C. The maximum point of heat absorption occurred at 38.24 minutes, at a temperature of 410.13°C. As a result of this exothermic effect, 1.82 J (398.65 J/g), or 409.45 µcal (94.08 cal/g) of heat was released. This heat release was attributed to the decomposition of the organic part of the sample. The Thermogravimetric Analysis (TGA) of the obtained sample showed that thermal decomposition proceeded in three distinct stages. The first stage of mass loss began at 0.05 minutes into heating, when the temperature reached 36.92°C, and ended at 18.87 minutes, at a temperature of 116.76°C. During this stage, 2.101 mg, or 17.258% of the total mass, was lost. This mass loss in the specified temperature range is associated with the removal of hygroscopic water and the partial decomposition of organic components. The second stage of significant mass loss began at a temperature of 190.82°C and ended at 346.30°C. This process started at 18.87 minutes into the heating and concluded at 31.75 minutes. During this stage, 0.233 mg of the sample was decomposed, corresponding to 1.914% of the total mass. The third stage of mass loss began at 31.85 minutes and continued until 38.24 minutes. This stage started at 410.13°C and lasted until the temperature reached 800°C. In this phase, 0.193 mg of the substance, or 1.585% of the total sample mass, was decomposed. Up to approximately 305°C, all organic components present in the sample underwent complete thermal decomposition. Based on the results of the Differential Thermal Analysis (DTA) and Thermogravimetric Analysis (TGA), it can be concluded that the obtained semiconductor pigment exhibits high thermal stability, as indicated by the relatively low total mass loss.

4.4. Elemental Fluorescence Analysis of Cobalt Phthalocyanine Pigment

Elemental fluorescence analysis is a highly effective analytical method for identifying and quantifying the components of a substance by measuring the characteristic radiation emitted by its elements. In this technique, the sample is first irradiated with high-energy light, causing each element within the material to emit its own specific fluorescence radiation. By analyzing this emitted radiation, information regarding the elemental composition and the relative quantities of those elements can be obtained. This analytical method is widely used due to its high precision and rapid results in determining the chemical composition of materials. Using this method, the specific fluorescence emissions of the cobalt-containing pigment were measured, enabling the identification and quantification of its constituent elements [19].

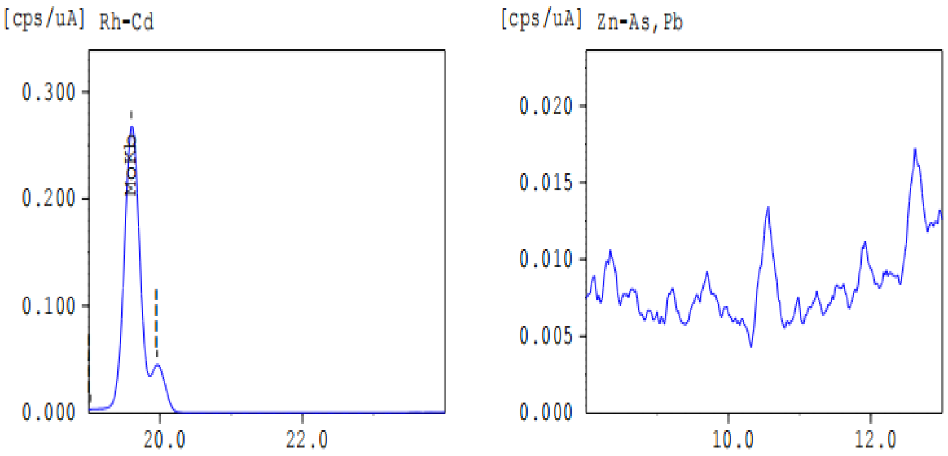


Fig. 5 Elemental fluorescence analysis of cobalt phthalocyanine pigment

The elemental composition of a substance must be known in order to control raw materials, production processes, and the final products in any manufacturing setting. Taking this into account, low-concentration solutions of the pigment were prepared in an organic solvent, and elemental analysis was carried out. When analyzing these low-concentration solutions, in addition to the cobalt-containing phthalocyanine dye, the absorption peaks revealed trace amounts of molybdenum likely originating from the catalyst used in the reaction and calcium residues remaining from the neutralizing agent. These were present within the margin of experimental error and indicate incomplete purification of the product.

4.5. Photodynamic Properties Analysis of Cobalt Phthalocyanine Pigment

To investigate the photodynamic properties of the cobalt-containing phthalocyanine dye, it is necessary to analyze its spectral absorption characteristics. In the present study, the optical absorption of the cobalt-containing phthalocyanine dye was examined using a V-5000 spectrophotometer across a spectral range of 350 nm to 1100 nm. The analysis was performed using a 1% solution of the dye in dimethylformamide. Figure 5 shows the optical absorption spectrum of the cobalt-containing phthalocyanine dye measured relative to the solvent across the specified spectral range. The optical absorption peaks of the obtained dye exhibited characteristic bands with high absorption intensity in the 700–800 nm range and lower intensity in the 500–600 nm range. The remarkable chemical and photophysical properties of phthalocyanines arise from their multi-electron system. The strong optical absorption bands result from π–π* electronic transitions between the Highest Occupied Molecular Orbital 9 HOMO) and Lowest Unoccupied Molecular Orbital (LUMO) energy levels [20].

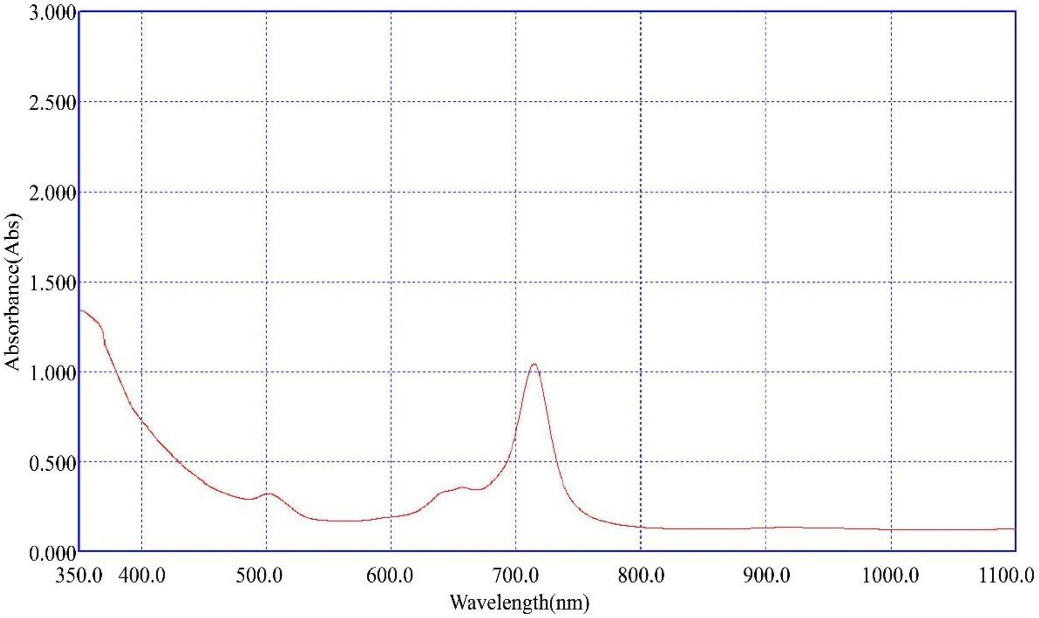


Fig. 6 Optical absorption analysis of cobalt phthalocyanine pigment

4.6. Absorption of Electromagnetic Radiation by Phthalocyanines in the Ultraviolet and Visible Regions

The absorption spectra of metallophthalocyanines exhibit two main and highly intense bands. The first is the Soret band, located in the 300–400 nm range, which corresponds to a high-energy and strong absorption peak in the ultraviolet (blue) region. The second is the Q-band, found in the 650–800 nm range, representing a low-energy absorption that defines the pigment's color, typically observed in the red to blue region of the visible spectrum [21]. The shape and position of the peaks in the absorption Spectrum can vary depending on the nature of the substituents in the central and peripheral positions of the phthalocyanine molecule, its solubility in different solvents, and the specific molecular structure. Moreover, the ligand π-orbitals can act as both π-donors and π-acceptors. The pyrrole nitrogen atom orbitals determine the coordination with the central metal atom, and together with the π-orbitals, they form a conjugated electron system. The graph demonstrates shifts in spectral peaks and differences in intensity under the influence of various solvents.

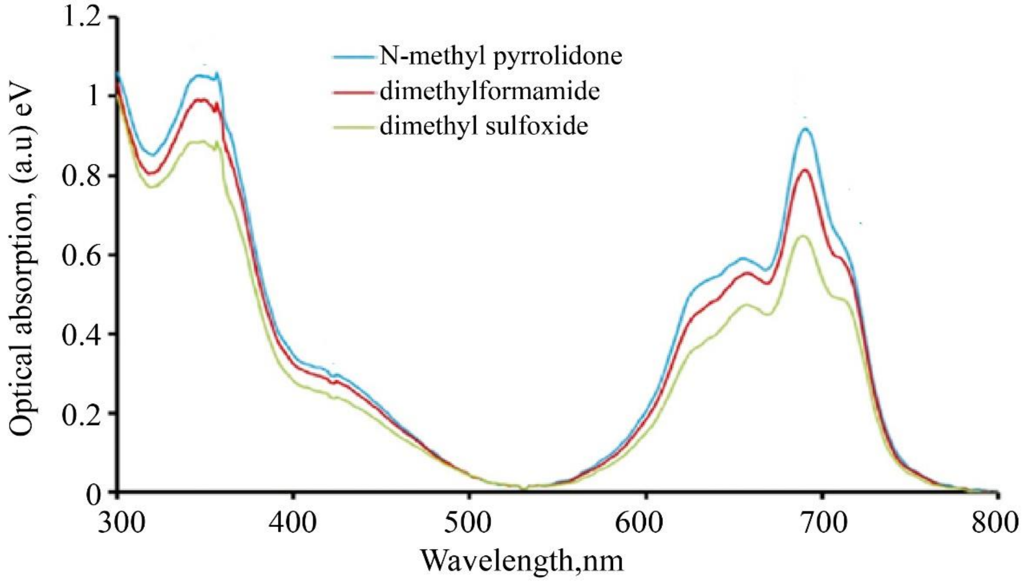


Fig. 7 Optical absorption spectrum analysis of cobalt phthalocyanine pigment

4.7. Estimated Optical Absorption Spectra of Cobalt Phthalocyanine Pigment Dissolved in Various Organic Solvents

The graph provided illustrates the shifts and intensity differences in the absorption peaks of cobalt phthalocyanine under the influence of various solvents. In N-methylpyrrolidone, the Soret band (a high-energy, strong absorption peak) appears around 340 nm, while the Q-band (a low-energy absorption region) is observed at 675 nm. In dimethylformamide, the Soret band is located near 345 nm, and the Q-band is observed around 665 nm. In dimethyl sulfoxide, the Soret band appears at approximately 335 nm, and the Q-band is found near 640 nm. These two optical absorption bands are critical indicators in studying the photophysical and photochemical properties of phthalocyanines. Moreover, many PcM (metal phthalocyanines) spectra exhibit three additional absorption bands in the Ultraviolet (UV) region, designated as follows:

N-band – 36,400 cm^{-1} (275 nm),

L-band – 40,800 cm^{-1} (245 nm),

C-band – 47,600 cm^{-1} (210 nm).

The N-band is particularly sensitive to structural changes, especially those related to the substitution of the central metal atom. In the previously mentioned B (Soret) and Q absorption regions, the most intense and weakest bands correspond to electronic transitions between the Highest Occupied Molecular Orbital (HOMO) and the Lowest Unoccupied Molecular Orbital (LUMO). The energy difference between these frontier orbitals, commonly known as the HOMO–LUMO gap, governs the optical behavior of metal phthalocyanines under incident light. This gap also provides critical insight into the pigment's intensity, chemical stability, and the anticipated coloration of the compound in solution [22].

4.8. Photovoltaic Parameters of Cobalt Phthalocyanine Dye and Natural Dye-Based DSSCs

For the first time, a cobalt-containing phthalocyanine dye pigment was applied in solution to dye-sensitized solar cells. The optical and semiconducting properties and the current and voltage values were studied for 10 days. Below are the values obtained by measuring the power output (mV) and (mA) for 10 days using a contact formed on the basis of raspberry fruit extract with a cobalt-containing phthalocyanine dye. (Table 2.) During the testing of the light absorption properties of phthalocyanine-based dye molecules, a 5 % solution of a cobalt-containing phthalocyanine dye was used. The power output, i.e., voltage and current values, was measured by contacting a solution of a cobalt-containing semiconductor dye in n-methyl pyrrolidone using a special glass plate with one side transparent.

Table 2. Voltage and current values generated by raspberry fruit extract with cobalt phthalocyanine used as a dye in solar cells based on solar-sensitive dyes

Cobalt phthalocyanine-based dye			Natural dye made from raspberry meba extract		
№ Time, day	**Voltage, (mV)**	**Current, (mA)**	**№ Time, day**	**Voltage, (mV)**	**Current, (mA)**
1	885.6	95.4	1	490	68
2	880,3	90.1	2	485.6	66.1
3	845	88,2	3	460.1	64
4	765	79.6	4	435.8	58.3
5	746	72.5	5	412.4	46.7
6	724	70.7	6	390.5	39.2
7	712,6	63.9	7	378.6	30.9
8	676	58.3	8	350.9	27.5
9	660	45.6	9	346	24.9
10	612	40.8	10	343	22.6

For comparison, the voltage and current values obtained in solar cells using a cobalt phthalocyanine-based dye and a sensitive natural dye prepared from raspberry fruit extract were compared. As a result, the highest values obtained on the first day of measurement were recorded as current 885.6 (mV) and voltage 95.4 (mA), and the lowest values obtained on the last day of measurement were recorded as voltage 612 (mV) and current 40.8 (mA).

Also, the highest values obtained on the first day of measurement were recorded as current 490 (mV) and voltage 68 (mA), and the lowest values obtained on the last day of measurement were recorded as current 343 (mV) and voltage 22.6 (mA). It is evident that the synthesized sunlight-sensitive cobalt phthalocyanine dye pigment is more stable to aggressive environments than natural raspberry fruit extract, and the generated voltage and current values are found to be significantly higher.

5. Conclusion

A cobalt-containing phthalocyanine pigment sensitive to sunlight was synthesized. The obtained semiconductor cobalt phthalocyanine pigment was analyzed using elemental fluorescence analysis and Raman spectroscopic analysis methods, as well as by comparison with existing phthalocyanines. Also, the quantum-chemical properties of the isoindole ligand in this phthalocyanine pigment were analyzed based on density functional theory.

According to the results of Differential Thermal Analysis (DTA) and Thermogravimetric Analysis (TGA), the synthesized pigment is thermally stable. In addition, the obtained cobalt phthalocyanine pigment was dissolved in various solvents, and its optical absorption spectra, i.e., photodynamic properties, were studied in a UV spectrophotometer. To determine the voltage (mV) and amperage (mA cm^{-2}) values, sensitivity was measured for 10 days under a 320 mW/cm^2 light source. Due to the good ability of this pigment to absorb photons from sunlight, the maximum voltage of 885 (mV), minimum 612 (mV), maximum current of 95.4 (mA), and minimum current of 40.8 (mA) were recorded in dye-sensitized solar cells. The synthesized photoconductive dye showed the characteristics of semiconducting dye pigments in terms of its excellent optical and photochemical behavior.

Based on the analysis of the volt-ampere characteristics of cobalt phthalocyanine, it was proven that it can be used as a sunlight-sensitive dye in the creation of new-generation solar cells.

Ongoing intensive research is aimed at expanding the application of cobalt-containing phthalocyanine pigments in solar cells, photoanodes, and energy storage systems in the future. These efforts will contribute to sustainable development, environmental balance, and energy security.

5.1. Recommendations

Due to the pigment's excellent ability to absorb photons from sunlight, it is recommended as a semiconducting sensitizing dye pigment for dye-sensitized solar cells. The results obtained provide a deep understanding of the electronic structure of phthalocyanine-based metal complexes and expand the scope of applications of such compounds as sensitive dye-pigment materials for optoelectronics, catalysis, sensors, and solar cells. These approaches also serve as a reliable scientific basis for future experimental studies.

Acknowledgments

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